Community Dentistry Oral Epidemiology

Prevalence and extent of enamel defects in the permanent teeth of 8-year-old Nigerian children.

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Abstract

Objectives: Enamel formation is a vulnerable developmental process, susceptible to environmental influences such as excessive systemic fluoride (F) exposure and infant/childhood disease. This study determined prevalence and extent of developmental enamel defects (DDE) and dental fluorosis in 8-year-old Nigerians and explored associations with key predictors.

Methods: A sample of 322 healthy 8-year-olds (155 males, 167 females) from primary schools in lower and higher water F areas of; i) rural and ii) urban parts of Oyo State in south-west Nigeria (n=4 areas) (in which the mean (SD) F concentration of community water supplies ranged from 0.07 (0.02) – 2.13 (0.64) mg F/L), were dentally examined using modified DDE (mDDE) and Thylstrup and Fejerskov (TF) indices. Drinking waters, cooking waters and toothpaste samples were analysed for F concentration using a F-Ion Selective Electrode (F-ISE). Information on infant/childhood diseases, infant feeding and tooth cleaning practices was obtained from parents/legal guardians. Data were analysed using ANOVA, Chi Square tests, Spearman correlation and binary logistic regression as appropriate.

Results: Mean (SD) F concentration of actual drinking and actual cooking waters consumed by participants were 0.25 (0.20) and 0.24 (0.14) mg F/L respectively in the urban higher F area; 1.11 (1.00) and 1.16 (1.02) mg F/L respectively in the rural higher F area ($p<0.05$). Overall, mouth prevalence of DDE in the permanent dentition was 61.2% with a mean (SD) of 2.4 (2.2) index teeth affected. Dental fluorosis mouth prevalence was 29.8% with a mean of 2.1 (3.7) teeth affected. Prevalence and extent of DDE and dental fluorosis was greater in higher F than lower water F areas ($p<0.001$). A weak positive correlation was seen between extent of dental fluorosis and drinking water F concentration (ρ =0.28). The absence of infant/childhood disease was associated with a lower risk of DDE being present (p=0.001), with an Odds Ratio of 0.43 (95% CI=0.26, 0.71). Gender was a statistically significant (p=0.014) predictor for dental fluorosis with females having a higher risk (OR 1.94 (95% CI=1.14, 3.28) of dental fluorosis than males.

Conclusions: In these Nigerian 8-year-olds (n=322), mouth prevalence of DDE was 61.2% (mean (SD) teeth affected = 2.4 (2.2)) and a key positive predictor was a history of infant/childhood disease. With 29.8% of these children exhibiting dental fluorosis (mean

(SD) teeth affected = 2.1(3.7)), drinking water F concentration was identified as a positive predictor, along with gender, with females more at risk of dental fluorosis than males.

Introduction

Insults to the enamel organ during enamel formation can result in aberrations in the quality and quantity of enamel which can present as enamel hypoplasias or enamel opacities, including dental fluorosis[1]. Several studies on the prevalence and severity of developmental defects of enamel (DDE) and associated risk factors have been undertaken in different parts of the world but limited work in Nigeria has shown a range in prevalence of DDE, from 11% reported for 4–16 year-olds[2] to 23% reported for 12 year-olds[3] residing in 0.15 – 1.41 ppm F areas[4], while dental fluorosis has been reported as ranging from 13%[5] to 51%[6] in 12–15 year-olds living in 0.59–0.75 ppm F areas and 0 - 0.4 ppm F areas respectively. The actual prevalence and extent of DDEs can differ widely among different populations due to the relative impact of different aetiological and associated risk factors, but the reported prevalence of DDE also varies widely, in part because of the different terminologies and diagnostic criteria used in measurement indices. Developmental enamel defects can predispose to aesthetic problems[7] usually, but not always, when there is loss of tooth structure, as in hypoplasia or severe dental fluorosis. Any loss of tooth structure increases the risk of early childhood caries[8] and attrition[9] and can lead to common subsequent complications including occlusal dysfunction and tooth sensitivity[7, 10]. The sensitivity and pain from these teeth may make children uncooperative during treatment of the defects[7] and their presence may create difficulties with anaesthetizing teeth and bonding of restorations to the enamel[7].

In view of these challenges, it is important to assess the extent of these dental conditions in a population in order to be able to determine the best ways to manage them with the resources available, particularly when these are limited. The use of standardized indices for measurement is also necessary to enable comparisons among different populations, and also within populations following preventive interventions. Most important though, is to determine the main risk factors for development of these conditions to help prevent them, or at least mitigate their impact where possible. Many factors including childhood disease[8], birth conditions[9], nutritional status[10], excessive fluoride intake during tooth development and genetics[11] have been implicated, in aetiological terms, in a greater risk of occurrence of

enamel defects, but valid and reliable evidence for the involvement of these factors remains elusive suggesting a need for further investigation. In settings like sub-saharan Africa, where children can be exposed to a number of risk factors for enamel defects, their presence and the associated increased risk of early childhood caries, tooth sensitivity, attrition and occlusal dysfunction can all impact on quality of life as well as the resources required for their subsequent management. These are important considerations when developing strategies and policies to optimise childrens' oral and general health, especially in developing countries.

As part of a larger study into factors associated with the occurrence of enamel defects in 4- and 8-year old children in Nigeria (which included the estimation of F intake, excretion and body retention in a sub-sample of the present sample of children), the aims of this study were, first, to determine the prevalence and extent of developmental enamel defects, including dental fluorosis in permanent teeth of 8-year-old Nigerian children and second, to identify putative predictors for their occurrence.

Methods

The study protocol was approved by the Ethics Committee, Newcastle University, UK and the University of Ibadan Ethical Review Board and undertaken between February (end of dry season) and July (mid rainy season) 2013 in 16 primary schools in Oyo South Senatorial district of Oyo State, south-west Nigeria following permission from the Ministry of Education, Oyo State. The four study locations were chosen by randomly selecting two Local Government Areas (LGAs) - one urban (Ibadan North with a population of 306,795) and one rural (Ibarapa Central with a population of 102,979) – from the total of 33 LGAs in Oyo State (population 5.6 million), Nigeria[12]. All common community ground water sources in these two LGAs were then identified locally by talking to opinion leaders who had resided locally since birth and who knew which water sources were used by the public. Water samples from each of the 124 groundwater sources identified across rural and urban sites in these two LGAS, were analysed for F concentration (mg/L ≡ ppm) in a laboratory at the University of Ibadan, using a F-Ion-Selective-Electrode (Model 9409 Thermo Orion, USA) and meter (Model 720), directly, after adding TISAB III[13].

Based on the F analyses of all these common community ground waters, four water F areas within the two LGAs were identified as urban higher (0.85ppm F), rural higher (2.13ppm F),

urban lower (0.07ppm F) or rural lower (0.09ppm F) water F areas and these four areas formed the settings in which the study was subsequently undertaken in the local primary schools. Additional community water samples collected from 24 sites across these 4 areas were then analysed for F concentration (Table 1) to determine the range of F concentrations in community water supplies in each of the four study locations.

For determining the sample size, data from a total of 322 eight year-olds were estimated as being necessary, based on a power of 95% at an alpha level of 5%, to determine a difference in mouth prevalence of DDE or dental fluorosis of 3% between areas and with an expected non-completion rate of 30%. Cluster sampling of healthy 8-year-olds of both genders was then undertaken, with primary schools in these 4 locations as clusters. A clinical dental examination of the mixed dentition was undertaken using a wooden spatula, dry gauze and a disposable mouth mirror (DenLite Illuminated Dental Mirror, Miltex Inc. USA). The dmft/DMFT index was used to record caries experience and a modified DDE index[14] to record developmental defects of enamel, while dental fluorosis was recorded using the TF Index[15]. This dental examination was carried out by a dentist (OI) who had been trained and calibrated in the diagnosis of dental caries, DDE and dental fluorosis with the support of appropriate reference and calibration materials[15, 16]. A random sample of 37 (11.5%) participants was re-examined for all dental indices and intra-examiner reproducibility determined.

Information about participants' toothbrushing behaviour and feeding habits during infancy and early childhood, as well as their history of infant/childhood diseases, was obtained from parents and guardians using an interviewer-administered questionnaire developed from a standard questionnaire[17], and translated into the local language (Yoruba). Prior to administering, the developed questionnaire was pre-tested among mothers with similar socio-demographic characteristics as the mothers of study participants and the local language wording was modified to ensure that it retained its reliability and validity. Samples of drinking and cooking waters consumed by the children were also obtained from their parents/carers. Toothpastes identified as being used by the children were purchased from local shops and transported to Newcastle University (UK) for F analysis. A Fluoride-Ion Selective Electrode (F-ISE) with a direct method[13] was used to assay F in waters, and also in toothpaste samples following pre-treatment[18]. Observational studies, mainly in children younger than 7 years of age, show a wide range in percentage of toothpaste swallowed per brushing, ranging from 12% to 84% [19]. In view of the scarcity of global data for 8-year-olds

and lack of any data from Nigeria, data from Iran and the UK were used to estimate the mean proportion of toothpaste ingested per brushing. A proportion of 41% - estimated for 4-year-olds in Iran, a developing country[20] as well as for 4-6-year-olds in the UK, a developed country[19] - was used to estimate F intake from toothpaste ingestion. Therefore, in the current study, to estimate inadvertent ingestion of F from routine toothpaste use, dispensed amounts of toothpaste were recorded by pictorial scale, multiplied by the toothpaste's F concentration (μg/g) and frequency of daily use and then multiplied by 41%. The overall daily F intake from toothbrushing for each child was then estimated in mg/day and on a body weight basis (mg/kg bw/day).

The intra-examiner variability was determined by kappa statistics. Using SPSS[21] a descriptive analysis of age and gender of study participants, water F concentrations, presence and extent (no. of teeth affected) of enamel defects, dental fluorosis and caries experience was generated and then a chi-square test used to test associations between categorical variables, while one-way ANOVA was used to compare means of more than 2 groups at $p<0.05$. After F analysis, results for actual drinking and cooking waters consumed were stratified according to their F concentrations into 3 groups (<0.7 ppm F, 0.7-1.2 ppm F and >1.2 ppm F) and their correlation with the extent of dental fluorosis seen in participants explored. The associations between the dichotomous dependent variables (DDE 1-8: yes/no and TFI>0 yes/no) and the explanatory independent variables were then modelled using binary logistic regression analysis all independent variables entered into the model at the same time and with the statistical significance level set at $p<0.05$.

Results

Overall, the mean (SD) age and weight of the 322 study participants were 8.5 (0.3) years and 22.31 (3.15) kg respectively. Males comprised 48.1% of the sample across the 4 areas with no statistically significant differences in age, weight or gender by area.

As Table 1 shows, the mean (SD) F concentration of community ground water supplies ranged from 0.07 (0.02) mg F/L in the urban lower F area to 2.13 (0.64) mg F/L in the rural higher F area. However, the mean (SD) F concentration of actually consumed drinking waters ranged from 0.25 (0.20) mg F/L in the urban higher F area to 1.11 (1.00) mg F/L in the rural higher F area ($p<0.05$). For the actually consumed cooking waters, the range of F concentrations was similar; from 0.24 (0.14) mg F/L in the urban higher F area to 1.16 (1.02) mg F/L in the rural higher F area ($p<0.05$).

In the primary dentition, the overall caries prevalence was 16.8%, with a range from 2.4% in the rural lower F area to 35.8% in the urban higher F area ($p<0.001$). For the permanent dentition, the prevalence of caries ranged from 0% in the rural lower F area to 13.6% in an urban higher F area ($p<0.001$). In terms of the extent of caries experience, the mean dmft ranged from 0.1 (0.5) in the rural lower F area to 1.0 (1.7) in the urban higher F area ($p<0.001$), while the mean DMFT ranged from 0.0 in the rural lower F area to 0.3 (0.7) in the urban higher F area ($p<0.001$).

The Kappa value for intra-examiner agreement in the recording of presence or absence of DDE, using the modified DDE index, for the 11.5% (n=37) of participants re-examined was 0.892 ($p<0.001$) showing excellent agreement, while for the TF index, the Kappa score was 0.840 ($p<0.001$) also indicating excellent agreement. Overall, 17.5% of the children with a DDE score >0 had 1 tooth affected by DDE, the remaining 82.5% of these children having 2 or more teeth affected. As Table 2 shows, the mouth prevalence of DDE ranged from 37.3% of participants in the rural lower F area to 83.5% in the rural higher F area ($p<0.001$) with the mean (SD) number of affected index teeth in the permanent dentition being lower ($p<0.001$) at 1.2 (1.6) in the rural lower F area compared to the rural higher F area (3.9 (2.2)). Overall, across all 4 areas, diffuse opacities were the most commonly recorded DDE, with a mouth prevalence of 6.2%, 9.9% and 41.6% respectively, reported for hypoplasia only, demarcated opacities only and diffuse opacities only in the permanent dentition (Table 2). Tooth prevalence across all 4 areas showed that 706 (43.1%) of the 1655 index teeth in the permanent dentition examined for DDE had developmental defects, with 80.3% of these

affected teeth having diffuse opacities, either alone (65.3%), in combination with demarcated opacities (2.3%) or with hypoplasias (11.9%), or in combination with both demarcated opacities and hypoplasias (0.8%) (Data not shown). In terms of the TFI measurement used to assess dental fluorosis, overall, 99% of the 96 children with a TFI >0 had 2 or more permanent teeth affected. Table 2 shows that dental fluorosis was significantly less prevalent (5.1%) in the urban lower F area than in the rural higher F area (82.3%) (Tukey post hoc test; $p<0.001$).

When the association between the extent of dental fluorosis in permanent teeth and F concentration of the actual drinking and cooking waters consumed by these 8-year-olds was explored (Table 3), the correlation for all areas overall was low, and the relationship between the F concentration of drinking waters and extent of dental fluorosis in permanent teeth was weak and positive (Spearman Correlation Coefficient = 0.281).

Gender was a statistically significant ($p=0.014$) predictor for dental fluorosis with females having a higher risk (OR 1.94 (95% CI=1.14, 3.28) of dental fluorosis than males.

As Table 4 shows, the absence of infant/childhood disease was associated with a lower risk of DDE being present (B coefficient was -0.83, Odds Ratio was 0.43 (95% CI=0.26, 0.71)). No other explanatory variables were associated with DDE. The binary regression model was able to make a correct prediction for 64.8% of the children having DDE or not. The Nagelkerke R^2 value (similar to the R^2 used in linear regression, and it provides a statistical measure of how well the independent variable(s) account for the dependent variable) from the binary logistic regression model was 0.11 and 0.12 for DDE and dental fluorosis respectively, indicating that 11% and 12% of the variability in the occurrence of DDE and dental fluorosis respectively was accounted for by the independent variables. When the association between dental fluorosis and the independent variables was modelled, gender was the only statistically significant predictor (Table 5). Females had a higher risk of dental fluorosis in the permanent dentition being present at age 8 years (Odds Ratio = 1.94; B coefficient = +0.66; 95% CI; 1.14, 3.28; $p=0.014$) and the binary regression model was able to make a correct prediction for 71.1% of the children having dental fluorosis or not**.**

Discussion

Some Nigerian studies have reported the prevalence of DDE[2, 3] and dental fluorosis[5, 6, 22] among children but not all have reported the water F concentration in the study environment. Tooth prevalence of DDE was not reported in previous Nigerian studies, therefore this present study is the first to report on the range and extent of enamel defects. These reporting differences are important considerations when trying to determine the major causes and associated risk factors for these conditions, since excessive F in drinking and cooking waters is well recognised as an important risk factor for dental fluorosis. This present study also reports, for the first time, the association between estimates of F exposure from waters and toothpaste, as well as other possible risk factors associated with DDE occurrence in Nigeria.

In terms of study limitations, these relate mainly to the difficulty in collecting data relating to historical exposure to F, in particular, during the early childhood window of susceptibility for DDE and dental fluorosis of aesthetically important teeth, especially if the F concentration of a water source (or the water source itself) has changed during a childhood so that current water sources are significantly different to those used in the earlier years of tooth development[17]. Recall of facts when historical data are being sought around early childhood disease and toothbrushing habits can also limit the validity of data collection. In general, a holistic approach to the understanding of F exposure from waters and toothpastes and other risk factors for DDE is important when trying to mitigate against or prevent these conditions in planning and making public and dental health policy decisions. This is important, not only in Nigeria but in other sub-Saharan countries where, for practical reasons, body F burden cannot be sufficiently regulated and there may be inadequate prevention strategies against general and oral childhood diseases and conditions.

The association between enamel defects and potential aetiological factors is often difficult to establish when studies in different settings are compared, due to the different assessment indices employed[23] highlighting the need for a consensus agreement on the use of standardised indices to allow accurate comparison between studies. In this present study, the appropriate indices listed in 'WHO Basic Methods for Oral Health Surveys[16] and incorporated in many epidemiological surveys were used. The mDDE index, based on the type and appearance of the enamel defects was used to measure DDE. This index is descriptive and records both F and non-F-induced defects, and so it allows for the determination of the overall prevalence of enamel defects, including those associated with

dental fluorosis. In addition, the presence of dental fluorosis among study participants was assessed using the Thystrup and Fejerskov index, a popular and widely used index that scores enamel defects of fluorotic origin based on their clinical and histological appearance. The reasons for the differences in mouth prevalences of dental fluorosis recorded using the TFI compared with the F induced categories (diffuse opacities) of the DDE index are unclear but most likely relate to a difference in the threshold level between the "normal" and "affected" appearance of a tooth surface when using these indices. These differences do further highlight the need for a standardized approach using the same index and careful adherence to the index-specific criteria, when undertaking repeat observational surveys for F-induced defects of enamel in populations.

As described in Table 1, across the 4 study areas, the mean F concentration of the community ground water supplies ranged from 0.07 to 2.13 mg F/L, while the mean F concentration of actual drinking and cooking waters used by participants ranged from 0.25 to 1.11 mg F/L and 0.24 to 1.16 mg F/L respectively. This difference in F concentrations suggests that the water samples were collected from a number of different shallow wells and aquifers in the same locality, in agreement with a previous report from Iran[24] that also recorded a high variability in the F concentration in water obtained from shallow wells. In addition, some of this variability would have been due to seasonal differences, since the waters were sampled between February (end of dry season) and July (mid rainy season), and waters collected from shallow wells during the rainy season tend to be lower in F than those collected during dry season[25]. These seasonal differences in F concentrations of water supplies may be significant in terms of impact on daily F intake and would be a useful focus for future research. The mean (SD) F concentration of actual drinking and cooking waters consumed by children who lived in urban higher F areas was 0.25 mgF/L and 0.24 mgF/L respectively which did not reflect the mean F concentration of the local community water supply (1.00 mgF/L). In addition to samples of the actual drinking and cooking waters consumed being collected at a different times of the year and also being from other sources, within the community, use of different and distant aquifers as sources of drinking and cooking water from outside the immediate locality as well as the consumption of purchased waters rather than the local community water supply does highlight some of the challenges associated with provision of potable water supplies in general, but also the difficulties encountered when accounting for the F contents of drinking and cooking waters when multiple sources are commonly used. In addition, classifying an area for F exposure/risk by sampling the community water supply

might be inappropriate since this study showed that the F concentration of actual drinking and cooking waters consumed were often quite different from the F concentration of the community water supply. This is an area for which no comprehensive water source and volume consumption data exist in Nigeria and therefore would be a useful area for future research to understand (and help address) this basic need for reliable, practical and affordable sources of potable water.

The prevalence of DDE in the permanent dentition of participants shown in this present study was between 37.3% in the rural lower F area and 83.5% in the rural higher F area and illustrated the expected association between F induced diffuse opacities, and the concentration of F in community water supplies, since diffuse opacities accounted for the highest prevalence in terms of type of DDE, as seen in previous studies[26, 27].

Additional studies and further analyses of the existing data would be helpful to explore the risk factors associated with the different constituent categories of DDE in terms of F-induced and non-F-induced defects. The overall prevalence of DDE in permanent teeth in this present study (61.2%) falls within published mouth prevalences of 9.8% and 93% reported for 11 – 13-year-old Italians living in a 0.3ppmF water area[28] and 14-year-old Saudi Arabians living in a 2.71 ppm F water F area[10] respectively. However, when compared with data available in Nigeria, this prevalence was higher than the 11.7% and 23% reported for 10 – 19-year-old[29] and 12 year-old[3] Nigerians respectively living in 0.15-1.41 ppm F[4].

Globally, the prevalence of dental fluorosis in permanent teeth has been found to range from 4% reported for 12-year-old Lithuanians living in low water F areas (0.2 ppm)[30] to 100% among 10- and 15-year-old Kenyan children living in 2 ppm water F areas[11]. The range of dental fluorosis prevalence found in 8-year-olds in the current study (from 5.1% in the urban lower F area to 82.3% in the rural higher F area) falls within this global range but the fluorosis prevalence in the rural higher F areas was higher than the 12.9% reported for 12 – 15-year-old Nigerian[5] and 96.3% reported for 12-18-year-old Tanzanian[31] children respectively, living in ≥ 1.5 ppm F water areas. The relationship between drinking water F concentration and dental fluorosis has been studied around the world and a number of reports have discussed the African context[11]. Notably, some papers have reported a high prevalence of dental fluorosis even in areas with a low F drinking water concentration (< 0.5 mg/L) and this is thought to be due to the influence of F from other sources, or other influences (e.g. developmental or genetics)[6, 11]. In the present study, there was weak positive correlation

(ρ=0.281) between F concentration in drinking water and the extent of dental fluorosis in the permanent dentition of 8-year-olds though the correlation was weak. The presence of this relationship agrees with findings from previous studies in which the concentration of naturally found F in water is excessive[32, 33]. If adequate measures are not instituted to provide optimal F concentration in water especially in areas where the F concentration is currently excessive, fluorosis will continue to be an oral and general health problem for communities.

In the present study, the prevalence and extent of dental caries was low, in accordance with previous studies[34, 35] and lower in rural than urban areas, probably due to greater access to and consumption of cariogenic diets in urban environments.

Consistent with the findings from other studies, infectious diseases caused by bacteria and viruses during infancy and childhood[8, 36] were found to be associated with DDE prevalence in permanent teeth in this present study. In terms of infant health, there has been increasing evidence that birth weight/condition is associated with health outcomes in later life.[37, 38] Low birth weight has been reported to be related to increased rates of obesity, hypertension, hyperlipidemia, insulin resistance and type II diabetes in adults[38], and children with low birth weights or failure to thrive due to poor nutritional status or infant/childhood disease were found to have an increased risk of developmental defects of enamel, including hypoplasias[9,10]. In addition, it was observed that females were significantly more likely to develop dental fluorosis in their permanent teeth than males, although the mechanism for this association is not clear.

A female preponderance of having dental fluorosis has also been reported in previous studies,[39,40] while some other studies have reported that males were more likely to have dental fluorosis[32,33] and others have reported no gender differences[41,42]. One possibility for the greater likelihood of females developing dental fluorosis may be a shorter breast-feeding duration in girls than boys, reported in studies from North and Sub-Saharan Africa[43] and India[44], and consequently earlier exposure to excessive F from solid foods and/or drinks prepared with water. Further studies are required to investigate the association between gender and environmental factors in the occurrence of DDE and dental fluorosis.

Conclusions

In these Nigerian 8-year-olds (n=322), mouth prevalence of DDE was 61.2% (mean (SD) teeth affected = 2.4 (2.2)) and a key positive predictor was a history of infant/childhood disease. With 29.8% of these children exhibiting dental fluorosis (mean (SD) teeth affected = 2.1(3.7)), drinking water F concentration was identified as a positive predictor, along with gender, with females more at risk of dental fluorosis than males.

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Table legends.

Table 1: Mean (SD) fluoride concentration (mg/L) in community water (n=24), drinking water (n=319) and cooking water (n=319) samples for 8-year-old participants (n=322) by area.

Table 2: Mouth prevalence and extent (Mean (SD) no. of teeth affected) for Developmental Defects of Enamel (DDE score 1-8) and Dental Fluorosis (TFI>0) in permanent teeth and caries experience in primary and permanent teeth of 8-year-old participants by area. One or more teeth affected qualified as a Yes for both DDE and TFI.

Table 3: Correlation between F concentration (mg/L) in drinking and cooking water and the extent of dental fluorosis (no. of teeth affected) in permanent teeth of 8-year-old participants (n=322[1]). One or more teeth affected qualified as a Yes for dental fluorosis.

Table 4: Binary logistic regression analysis model for DDE (Yes/No) in permanent index teeth of 8-year-olds (n=322), where DDE score 1-8 = Yes and DDE score 0 = No. One or more teeth affected qualified as a Yes for presence of DDE.

Table 5: Binary logistic regression analysis model for dental fluorosis (Yes/No) in permanent teeth of 8-year-olds (n=322). One or more teeth affected qualified as a Yes for presence of dental fluorosis.

Table 1: Mean (SD) fluoride concentration (mg/L) in community water (n=24), drinking water (n=319) and cooking water (n=319) samples for 8-year-old participants (n=322) by area.

Water samples	**F concentration (mg F/L)**					**p value**	**Tukey Post-hoc following ANOVA**
	Urban, Higher F	**Rural, Higher F**	**Urban, Lower F**	**Rural, Lower F**	**All Areas (n=322)[1]**		
Community water supply							
Mean	0.85	2.13	0.07	0.09	0.84		
(SD)	(0.19)	(0.64)	(0.02)	(0.02)	(0.99)		
[n]	4	8	4	8	24		
Drinking water **actually consumed**							
Mean	0.25[a]	1.11[a]	0.75	0.27	0.72	<0.001	For [a] p=0.001
(SD)	(0.20)	(1.00)	(0.76)	(0.14)	(0.84)		
[n]	[80]	[78]	[78]	[83]	[319]		
Cooking water **actually consumed**							
Mean	0.24	1.16[a]	0.56	0.27[a]	0.67	<0.001	For [a] p<0.001
(SD)	(0.14)	(1.02)	(0.15)	(0.13)	(0.78)		
[n]	[80]	[78]	[78]	[83]	[319]		

[1] *Of the 322 eight-year-old children dentally examined, 319 provided drinking and cooking water samples;*

Table 2: Mouth prevalence and extent (Mean (SD) no. of teeth affected) for Developmental Defects of Enamel (DDE score 1-8) and Dental Fluorosis (TFI>0) in permanent teeth and caries experience in primary and permanent teeth of 8-year-old participants by area. One or more teeth affected qualified as a Yes for both DDE and TFI.

Enamel defects	Urban, Higher F (n=81)	Rural, Higher F (n = 79)	Urban, Lower F (n = 79)	Rural, Lower F (n = 83)	All areas (n = 322)	P values	Tukey Post Hoc Test following ANOVA
Developmental Defects of Enamel							
Mouth prevalence (DDE score 1-8). No. (%)	56 (69.1)	66 (83.5)	44 (55.7)	31 (37.3)	197 (61.2)	< 0.001[+]	
Demarcated opacities	8 (9.9)	4 (5.1)	14 (17.7)	6 (7.2)	32 (9.9)		
Diffuse opacities	42 (51.9)	60 (76.0)	20 (25.3)	14 (16.9)	134 (41.6)		
Hypoplasia	7 (8.6)	0 (0)	9 (11.4)	4 (4.8)	20 (6.2)		
Other defects	1 (1.2)	4 (5.1)	13 (16.5)	4 (4.8)	22 (6.8)		
Demarcated and diffuse opacities	1 (1.2)	2 (2.5)	1 (1.3)	3 (3.6)	7 (2.2)		
Demarcated opacities and hypoplasia	0 (0)	1 (1.3)	2 (2.6)	0 (0)	3 (0.9)		
Diffuse opacities and hypoplasia	15 (28.5)	10 (12.7)	7 (8.9)	5 (6.0)	37 (11.5)		
Demarcated & diffuse opacities and hypoplasia	0 (0)	0 (0)	0 (0)	1 (0)	1 (0.3)		
Extent (No. of index teeth affected). Mean (SD)	2.5[a,d] (2.0)	3.9[a,b,c] (2.2)	2.0[b] (2.3)	1.2[c,d] (1.6)	2.4 (2.2)	<0.001[#]	For [a,b,c,d] p<0.001
Dental fluorosis							
Mouth prevalence (TFI > 0). No. (%)	20 (24.7)	65 (82.3)	4 (5.1)	7 (8.4)	96 (29.8)	<0.001[+]	
Extent (No. of teeth affected). Mean (SD)	1.2[a] (2.6)	6.4[a,b,c] (4.0)	0.2[b] (1.3)	0.6[c] (2.3)	2.1 (3.7)	<0.001[#]	For [a,b,c] p<0.001
Dental caries experience							
Mouth prevalence (%) dmft >0	35.8	8.9	20.3	2.4	16.8	<0.001+	
DMFT >0	13.6	3.8	12.7	0.0	7.5	<0.001+	
Extent (No. teeth affected) Mean (SD)							
dmft	1.0(1.7)[a,b]	0.2(0.6)[a]	0.5(1.3)	0.1(0.5)[b]	0.4(1.2)	<0.001	For [a] and [b]; p<0.001
DMFT	0.3(0.7)[a]	0.1(0.3)	0.2(0.7)[b]	0.0(0.0)[a,b]	0.1(0.5)	<0.001	For [a]; p=0.01; [b] p= 0.04

[+] *Chi-square* [#] *One way ANOVA*

Table 3: Correlation between F concentration (mg/L) in drinking and cooking water and the extent of dental fluorosis (no. of teeth affected) in permanent teeth of 8-year-old participants (n=322[1]). One or more teeth affected qualified as a Yes for dental fluorosis.

Water F (mg/L)	n	Mouth prevalence of dental fluorosis No. (%)	No. of teeth affected Mean(SD)	ρ	P
<0.7					
Drinking water	283	70(24.7%)	2.8 (3.3)	0.173	0.003
Cooking water	281	69(24.6%)	1.7 (3.4)	0.021	0.723
0.7 – 1.2					
Drinking water	14	9(64.3%)	4.9 (4.4)	0.593	0.026
Cooking water	20	9(45.0%)	3.7 (4.6)	- 0.264	0.261
>1.2					
Drinking water	22	13(59.1%)	5.1 (4.9)	- 0.060	0.790
Cooking water	18	14(77.8%)	6.1 (3.8)	- 0.100	0.694
All areas					
Drinking water	319[1]	92(28.8%)	2.1 (3.7)	0.281	<0.001
Cooking water	319[1]	92(28.8%)	2.1 (3.7)	0.173	0.161

ρ = Spearman correlation coefficient. [1] Of the 322 eight-year-olds dentally examined, 319 provided drinking and cooking water samples

Table 4: Binary logistic regression analysis model for DDE (Yes/No) in permanent index teeth of 8-year-olds (n=322), where DDE score 1-8 = Yes and DDE score 0 = No. One or more teeth affected qualified as a Yes for presence of DDE.

Predictors	Developmental enamel defects (Yes/No) (R^2=0.11[a]; % Predicted =64.8%)				
	B	Sig (p)	OR [c] (Exp B)	95% CI Lower	Upper
Age (Years)	-0.46	0.272	0.63	0.28	1.44
Gender (Male/Female)	0.21	0.412	1.23	0.75	2.01
F Concentration Drinking Water (mg/L)	0.16	0.583	1.18	0.66	2.11
F Concentration Cooking Water (mg/L)	-0.22	0.464	0.80	0.45	1.44
Exclusive Breast Feeding (Yes/No)	0.78	0.387	2.17	0.37	12.61
Age Breast Feeding ceased (< />12 months)	1.26	0.123	3.52	0.71	17.42
Infant/childhood disease (No/Yes)	-0.83	**0.001[b]**	0.43	0.26	0.71
Age started tooth brushing (< 6 months)	-0.34	0.560	0.71	0.23	2.24
Age started tooth brushing (7-11 months)	-0.59	0.238	0.56	0.21	1.47
Age started tooth brushing (11-18 months)	-0.16	0.545	0.85	0.50	1.44
Frequency of tooth brushing (once/>once)	-0.82	0.421	0.44	0.06	3.24
Amount of toothpaste used per brushing (g)	1.03	0.533	2.79	0.11	70.05
Fluoride toothpaste ingestion (mg/day)	-0.68	0.637	0.51	0.03	8.44
Normal birth (No/Yes)	0.46	0.690	1.59	0.16	15.59
Family history - tooth discolouration (No/Yes)	0.91	0.084	2.49	0.88	7.01

[a] Nagelkerke R^2 [b] Statistically significant at P<0.05 [c] Odds Ratio

Table 5: Binary logistic regression analysis model for dental fluorosis (Yes/No) in permanent teeth of 8-year-olds (n=322). One or more teeth affected qualified as a Yes for presence of dental fluorosis.

Predictors	Dental fluorosis (Yes/No) (R^2=0.12[a]; % Predicted =71.1%)				
	B	Sig (p)	OR[c] (Exp B)	95% CI Lower	Upper
Age	0.86	0.059	2.37	0.97	5.81
Gender (Male/Female)	0.66	**0.014[b]**	1.94	1.14	3.28
F Concentration Drinking Water (mg/L)	0.45	0.131	1.57	0.87	2.81
F Concentration Cooking Water (mg/L)	-0.39	0.225	0.67	0.36	1.28
Exclusive Breast Feeding (Yes/No)	1.26	0.129	3.53	0.69	18.07
Age Breast Feeding ceased (</> 12 months)	0.35	0.602	1.42	0.38	5.26
Infant/childhood disease (No/Yes)	-0.13	0.632	0.88	0.52	1.48
Age tooth brushing started (< 6 months)	-1.03	0.200	0.36	0.07	1.72
Age of tooth brushing (7-11 months)	-0.69	0.266	0.50	0.15	1.69
Age of tooth brushing (11-18 months)	0.09	0.745	1.09	0.63	1.89
Frequency of tooth brushing (once/> once)	-0.44	0.614	0.64	0.11	3.61
Amount of toothpaste used per brushing (g)	-0.66	0.671	0.52	0.02	10.93
Fluoride Toothpaste ingestion (mg/day)	0.54	0.677	1.71	0.14	21.58
Normal birth (No/Yes)	1.61	0.108	4.99	0.70	35.49
Family history - tooth discolouration (No/Yes)	1.41	0.075	4.09	0.87	19.25

[a] *Nagelkerke R^2* [b] *Statistically significant at P<0.05* [c] *Odds Ratio*